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Herbicide Induced Reversion to Prototrophy of a Non-Sporulating Mutant of *Anabaena doliolum*

Anupma Srivastava[1] and A.K. Pandey[2]*

[1]Department of Botany, Shri A.K.A.P.G. College, Varanasi, India.
[2]Biological Research Lab, Department of Botany and Biotechnology, Kutir P.G. College, Chakkey - 222 146, Jaunpur, India.

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Spontaneous and MNNG-induced reversion of non-sporulating mutant (Ad-Ns, het$^+$ nif$^+$ sp$^-$) of the cyanobacterium *A. doliolum,* to prototrophy (Ad-S,het$^+$ nif$^+$ sport$^+$) was investigated to compare the mutagenic potential of the herbicide alachlor. Alachlor induced reversion to protrophy was greater than spontaneous and MNNG-induced frequencies. The prototrophic revertants, either spontaneous or MNNG and alachlor induced, of non-sporulating mutants have a heterocyst frequency of 5.1±0.6% as in parent. Sporulation started during stationary phase of the growth of the revertants (Ad-S, het$^+$ nif$^+$ sport$^+$) and spore frequency reached upto 92.0±0.25% against parents frequency (100%) the growth pattern of the revertant was slightly and significantly slower than the parent in N2-medium.

Key words: Cyanobacterium, Revertants, Prototrophy, Heterocyst, Sporulation, Herbicide.

A number of pesticides including herbicides should be properly evaluated for their possible mutagenicity before being recommended. A number of such agrochemicals when treated for their genetic toxicity in microbial and mammalian systems, have been proven to be highly mutagenic/ carcinogenic (Anderson and Anthony, 1972, Sharashu *et al.,* 1976, Pandey, 1999, Vishampayan *et al.,* 2000, Dwivedi *et al.,* 2007 a,b).

* To whom all correspondence should be addressed.
Mob.: + 91-9415652579, 9839187463
E-mail: ashokpandey_chakey@yahoo.co.in; ashokpandey233@rediffmail.com

In view of the importance of cyanobacteria in rice agriculture, an investigation for cyanobacteria is needed to assess the application of herbicide in rice field. Only few reports are available in the mutagenic potential of herbicides in cyanobacteria (Das and Singh, 1978, Vaishampayan *et al.,* 1998, 2000, Dwivedi *et al.,* 2007 a,b). The popular methods for detecting mutagenicity of such chemicals include either forward mutation test, leading loss of function of reverse mutation test for screening of their efficiency to reverse the stable auxotroph to prototrophy (Zimmerman, 1973, Ames *et al.,* 1973). The present work was therefore, undertaken to investigate alachlor (lasso) reversion of non-sporulating characters to prototrophic character and same were compared with frequency of spontaneous and MNNG induced mutation for the same manner.

MATERIAL AND METHODS

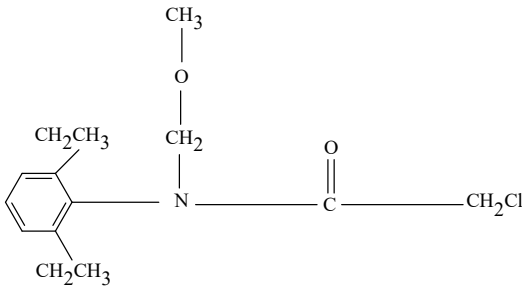
The clonal and axenic culture of a non-sporulating mutant (Ad-NS, het^+ nif^+ $spor^-$) and its parents strain (het^+ nif^+ spr^-) was routinely grown in Allen and Arnon growth medium (Allen and Arnon, 1995) at the expanse of elemental nitrogen. The culture was grown in a culture room at 28±1°C for 14 hrs photoperiod in cool day light fluorescent tubes of 2200 lux intensity.

Herbicide

Alachlor (lasso) or CP50144 is the trade name given to 2-chlor-2',6'-diethyl-N (methoxy methyl) acetanilide and acts as pre-emergence selective herbicide in rice field to control the grasses and broad leaf weeds.

Mutagenic reversion of protrophy experiment

Homogeneous suspension of exponentially grown culture was harvested by repeated centrifugation and washing with sterile



double distilled water. The cyanobacterial cells were homogeneously suspended in $N2^+$ medium and culture density of approximately 2×10^3 colony forming unit ml^{-1} were suspended in 200µg ml^{-1} alachlor and 100µg ml^{-1} MNNG treatment for 20 min and 30 min. respectively and regularly shaken during treatment period which permitted nearly 55% survival. The cultures were centrifuged and washed with sterile $N2^-$ medium and transferred to fresh medium separately. Treated and control samples were centrifuged after 48 hrs of incubation and harvested samples were plated on 1% agar containing $N2^-$ medium for isolation of prototrophic revertants, under induced and spontaneous conditions. Plates were incubated for a fortnight in growth chamber under earlier mentioned conditions. Revertants colonies were counted from each set of experiments. One of the revertant colony from each set picked up and raised separately into stock culture in $N2^-$ medium to compare with parent. Heterocyst and spore frequencies were counted by counting the number of heterocyst and spore per hundred vegetative cells.

RESULTS AND DISCUSSION

Genetic toxicity of the herbicide alachlor was evaluated for the frequency with which het^+ nif^+ spr^- (Ad-NS) strain of *A. doliolum* could revert to a het^+ nif^+ $spor^+$ (Ad-S) prototrophic character and compared with MNNG and spontaneous revertant frequencies. Selected doses and treatment duration period permitted 55% survival. The frequency of spontaneous revertants to prototrophy (het^+ nif^+ $spor^+$) was $1.5 \times 10^{-9} \pm 1.0 \times 10^{-10}$ while MNNG and alachlor induced reversion to prototrophy was $4.1\times10^{-3}\pm1.2\times10^{-4}$ and $3.95\times10^{-2}\pm 1.15\times10^{-3}$ respectively (Table 1). Frequency of spontaneous and induced reversion

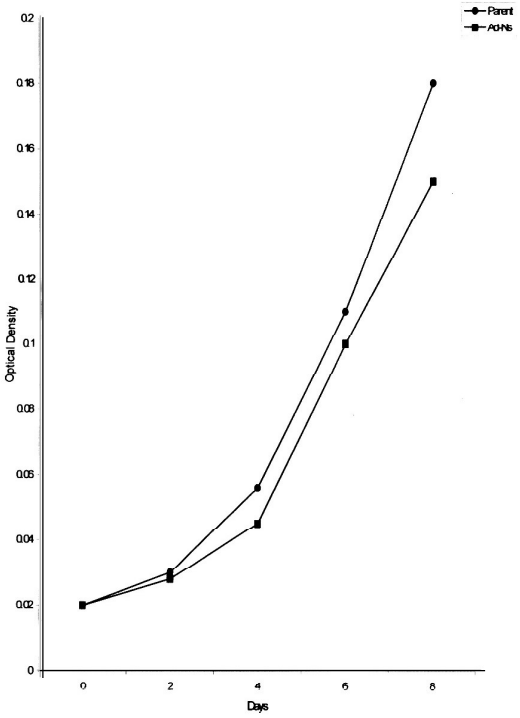


Fig.1. Comperative growth pattern of parent (-♦-♦-) & the revertant of Ad-Ns (-■-■-) in N2 Medium

Table 1. Surival, spontaneous and induced reversion frequency to prototrophy of non-sporulating mutants strain of *Anabaena doliolum* (Ad-NS) treated with alachlor (200 µgml^{-1} for 20 min) and MNNG (300 µm ml^{-1} for 30 min)

Strains	Percent surival		Frequency of reversion			
	MNNG	Alachlor	Spontaneous	MNNG induced	Alachlor induced	Phenotrypic constitution of revertant
Ad-Ns	553±1.35	55.0±1.67	$1.5\times10^{-9}\pm1.0\times10^{-10}$	$4.1\times10^{-3}\pm1.2\times10^{-4}$	$3.95\times1.15\times10^{-3}$	Ad-S*

*Ad-S *A. doliolium* (sporulating)

Table 2. Percent heterocyst and spore in parent and revertnat (Ad-S) of non-sporulating mutants (Ad-NS) of *A. doliolum* (Parent)

Strains	Frequency	
	Heterocyst	Spore
Parent	5.2±0.85	100
Ad-NS	5.1±0.70	-
Ad-S	5.1±0.65	90

to prototrophy clearly indicates that alachlor is mutagenic and induced mutation with a frequency almost comparable and significantly higher than induced by MNNG. The weak recombinogenic action of alachlor was reported in yeast cells (Gentle *et al.,* 1977), suggesting for action at genetic level. MNNG is known to induce morphological, biochemical, antibiotic and drug resistant mutant in cyanobacteria (Asato and Folsome, 1969, Kumar, 1975,Tiwari and Singh 1980) by acting on replicating DNA (Drake and Blatz 1976). Prototrophic reversion of *N. muscorum* (het$^+$ nif$^-$) to het$^+$ nif$^+$, after treatment with alachlor was reported by (Dwivedi *et al*., 2007 a b). Alachlor with alkyl group provides scope for its being equally effective that of MNNG in increasing mutation by mechanism known for alkylating agents (Singer, 1975, Vishampayan, 1984). Microscopic examination of 200 revertants colonies showed to be all sporulating. The prototrophic revertants, either spontaneous or by MNNG and alachlor induced, of non-sporulating mutant of *A. doliolum* (Ad-S) has a heterocyst frequency of 5.1 ±0.85% after five days (Table 2). Sporulation started during stationary phase of growth of the revertants (Ad-S ie. Sporulating) usually after 20 days and frequency reached upto 92.0±2.5% against parent frequency (100%) (Table 2). Growth pattern of revertants strains (Ad-S) was slightly slower than that of parent strain in N2-medium (Fig. 1). A slight and insignificant lower heterocyst frequency of the revertant against parent strain of revertants of het$^+$ nif $^+$ spor $^-$ seems to suggest its lower heterocyst frequency as a cause of its slower growth in N2$^-$ medium. The amide herbicide alachlor has been reported to inhibit gibberellic acid induced hydrolytic enzyme production in sensitive plants (Jawarski 1969, Devlin and Cunningham 1970).

CONCLUSION

On the basis of present observation it may be concluded that alachor, being a most potent toxic herbicide may cause mutation revertion of stable auxotrophic mutant to prototrophy. In view of the present results its increased discharged into the environment may lead to serious concern regarding the possible toxic hazards to living system.

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